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(54) Title: LIQUID STABLE COMPOSITION OF OXAZAPHOSPHORINE WITH MESNA

(57) Abstract: A low toxicity, stable oxazaphosphorine containing compositions with mesna for parenteral administration has been described. The process essentially requires addition of an oxazaphosphorine antineoplastic to the aqueous solution of an etherified b-cyclodextrin followed by addition of mesna as such or as an aqueous solution containing optionally, an etherified b-cyclodextrin. Preferably, the oxazaphosphorine antineoplastic is Ifosfamide and the etherified b-cyclodextrin is 2-hydroxypropyl-b-cyclodextrin.

LIQUID STABLE COMPOSITION OF OXAZAPHOSPHORINE WITH MESNA

Field of Invention

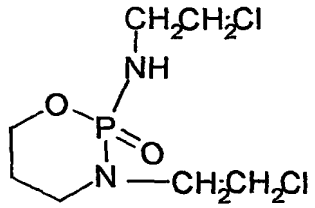
This invention relates to a process for preparation of low toxicity, stable aqueous ready-to-use oxazaphosphorine-containing compositions comprising an oxazaphosphorine antineoplastic, mesna and an etherified β-cyclodextrin. It has particular, but not exclusive, application to the preparation of compositions containing Ifosfamide, Mesna and 2-hydroxypropyl-β-cyclodextrin (referred to hereinafter as "HPBCD") suitable for parenteral administration in human beings and other mammals. The invention is more particularly related to a process for preparation of clear aqueous low toxicity compositions of Ifosfamide comprising Ifosfamide, Mesna, HPBCD that are stable over a period of time thereby making them suitable for ready clinical use.

Background of the invention

Two main groups of drugs used in the treatment of malignant disease are alkylating agents and the antimetabolites. Ifosfamide and cyclophosphamide are oxazaphosphorine antineoplastic drugs belonging to the alkylating agents group and are being widely used.

Ifosfamide is given intravenously either by injection as a solution diluted to less than 4% or by infusion and is used in the treatment of a variety of solid tumours including those of the cervix, endometrium, lung, ovary, testes and thymus as well as in sarcoma and in the treatment of Burkitts lymphoma.

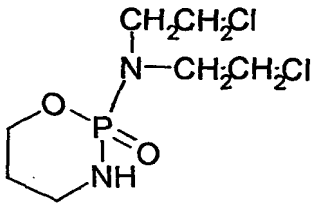
Ifosfamide is the Approved Name for 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-2H- 1,3,2-oxazaphosphorin-2-oxide and is represented by the formula:



It is a white hygroscopic crystalline powder having a low melting point of 40°C. It also begins to sinter below its melting point. These characteristics of Ifosfamide make it difficult for sterile filling of the dry powder as both temperature and humidity are required to be accurately controlled. Further, as Ifosfamide powder is filled aseptically into sterile containers, maximum precautions are required to maintain sterility of the product.

Ifosfamide powder is freely soluble in water. The aqueous solution is sensitive to changes in pH.

Similar problems are encountered with other oxazaphosphorine antineoplastic, e.g. Cyclophosphamide, which is the Approved Name for 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazophosphorine 2-oxide, represented by the formula:



Oxazaphosphorine antineoplastics are toxic to the urinary tract and may involve the kidneys as well as the bladder. Hence it is recommended that they are administered in association with 2-mercaptoethanesulphonates, especially Mesna. Mesna is the Approved Name for sodium 2-mercaptoethanesulphonate and is represented by the formula:

$HSCH_2CH_2SO_3^- Na^+$.

Mesna is highly water soluble. It is used for the prophylaxis of urothelial toxicity in patients being treated with Ifosfamide or Cyclophosphamide. In the kidney Mesna disulfide, the inactive metabolite of Mesna is reduced to free Mesna, which has thiol groups that react with the metabolites of Ifosfamide, and Cyclophosphamide, including acroleil, considered to be responsible for the toxic effects on the bladder.

The intravenous daily dose of Mesna is calculated to equal 60% of the total daily dose of Ifosfamide and is administered as 3 bolus doses given 15 minutes before and 4 and 8 hours after administration of each dose of ifosfamide when the ifosfamide dose is less than $2.5g/m^2$/day administered as a short infusion. For use with continuous infusion of Ifosfamide, Mesna may be administered as a bolus dose equal to 20% of the total ifosfamide dose followed by a continuous infusion of mesna equal to 40% of the ifosfamide dose, continuing for 12 to 24 hours after completion of the ifosfamide infusion.

Mesna has also been administered as a continuous infusion at a dose equal to 60% of Ifosfamide dose. No clinical data is available to justify Mesna doses greater than 60% w/w of Ifosfamide for standard doses of Ifosfamide. With high doses in excess of $2.5g/m^2$ of Ifosfamide, continuous and prolonged Mesna dosage regimen is necessary for maximum protection against urotoxicity.

The disadvantages with the existing commercially available product in powder form is that

1. more than one vial is required to be reconstituted and then diluted to the required concentration as the standard dosage is more than 1g daily.

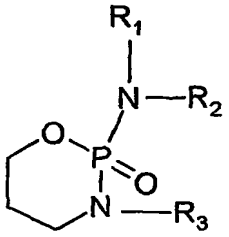
2. in high dosage Ifosfamide therapy as high as eight vials of 1g are required to be reconstituted and diluted to the required concentration.

3. as Mesna is required to be administered along with Ifosfamide, Ifosfamide solution after reconstitution is required to be mixed with Mesna.

Attempts were made by various laboratories / inventors to formulate ready-to-use parenteral solution that would contain Ifosfamide and Mesna to overcome the problem of handling Ifosfamide during reconstitution and during mixing with Mesna.

US-A-4959215 discloses a stable Ifosfamide-Mesna lyophilizate comprising Ifosfamide, 0.05 to 1.0 parts by weight of Mesna and 0.1 to 17 parts by weight of a hexitol prepared by freeze drying an aqueous or aqueous-ethanolic solution of Ifosfamide, Mesna and the hexitol, preferably mannitol. There is no reference to the presence of any cyclodextrin. The lyophilizate is stable physically showing no discolouration. The speed of dissolution is also claimed to markedly higher compared to the dry filled Ifosfamide.

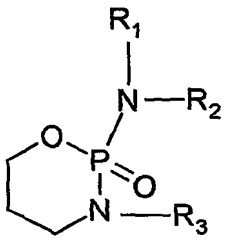
US-A-4952575 discloses a composition comprising 10 to 70 %w/v of an oxazaphosphorine of the formula:



in which at least two of R_1, R_2 and R_3 independently are 2-chloroethyl or 2-methanesulfonyloxyethyl and any remaining R radical is selected from hydrogen, a, methyl and ethyl,

dissolved in 80 to 100 %v/v of ethanol. Even though the degradation has been shown to be minimal for Ifosfamide, use of solvents in such a high concentration leads to other problems such as volatility, handling during manufacturing, miscibility with blood. As ethanol is pharmacologically active, this may also affect the person on administration of alcoholic solution of Ifosfamide.

WO-A-9918973 discloses stable ready-to-use liquid compositions of at least one oxazaphosphorine of the formula:



in which R_1, R_2 and R_3 independently are methyl, ethyl, 2-chloroethyl, 2-methanesulfonyloxyethyl or, except for R_3, hydrogen, and at least two of R_1, R_2 and R_3 are 2-chloroethyl and/or 2-methanesulfonyloxyethyl,
comprising a physiologically well-tolerated compound which forms chloride ions in aqueous solution. It is independently stated that the composition can include cyclodextrins, preferably α-cyclodextrins, or their ethoxylated derivatives as tonicity adjustment agents and Mesna but there is no exemplification of any composition containing beta cyclodextrin or Mesna.

US-A-4879286 discloses a storage-stable liquid oncolytic formulation of Cyclophosphamide formulated as a ready-to-dilute solution in a carrier comprising 50 to 100 % organic polyol selected from propylene glycol, polyethylene glycol and glycerol and 0 to 50 % water. The formulation may be used in combination with alcohols, such as 10 to 30% of ethanol (based on total weight of the formulation).

US-A-6407079 discloses that the water-solubility and stability of sparingly water-soluble or water-instable drugs are improved by the formation of inclusion compounds with partially etherified β-cyclodextrins of the formula:

(β-CD)OR

in which R are hydroxyalkyl groups with optionally some being alkyl groups,

and having a water-solubility of more than 1.8 g in 100 ml water. Preferably, R are selected from hydroxyethyl, hydroxypropyl or dihydroxypropyl groups. This patent shows use of cyclodextrins for dissolving sparingly water soluble / insoluble drugs and does not indicate its usefulness for water soluble materials like Ifosfamide and Mesna.

US-A-4727064 discloses that lipophilic drugs can be stabilized by solubilizing the drug into an intrinsically amorphous mixture of a water-soluble cyclodextrin derivative to form a solubilized cyclodextrin/drug complex and, optionally, freeze-drying or evaporating the resultant solubilized complex to provide a solid cyclodextrin/drug complex in powder form. The exemplified mixtures of cyclodextrin derivatives are obtained by non-selectively alkylating α-, β-, or γ-cyclodextrin using, for example, propylene oxide, glycidol, iodoacetamide, chloroacetate or 2-diethylaminoethylchloride. The cyclodextrins can be substituted by hydroxyalkyl carboxamide, diethylaminoethyl, carboxymethyl or carboxyamidomethyl and exemplified cyclodextrins include hydroxypropyl-β-cyclodexrin. This patent does not suggest use of cyclodextrins for water soluble materials like Ifosfamide and Mesna.

WO-A-0139749 discloses fast dissolving pharmaceutical compositions in solid dosage form with prolonged sweet taste comprising (a) at least one drug, (b) at least one water soluble sugar, (c) at least one non-sugar sweetener in normal fast release form and (d) at least one non-sugar sweetener in a mucoadhesive slow release form. Exemplified drugs include Ifosfamide and Mesna and exemplified mucoadhesive agents include cyclodextrins. There is no

exemplification of any composition containing two or more of Ifosfamide, Mesna and a cyclodextrin.

As Mesna is required to be given concurrently with each dose of Ifosfamide, in one aspect of the invention Ifosfamide and Mesna are combined in the same composition to avoid the inconvenience of administering Mesna separately. In another aspect of the invention Ifosfamide and Mesna are combined with HPBCD to give a stable composition so that the product is readily marketable and is convenient to use without the step of reconstitution and less handling. Surprisingly, the process of invention in which Ifosfamide, Mesna and HPBCD are combined has produced a composition having low toxicity also.

The main objective of this invention is thus to develop a process for preparing low toxicity, stable compositions of Ifosfamide comprising Ifosfamide, Mesna, HPBCD, with or without conventional parenteral additives, overcoming all the disadvantages of prior arts and make the composition suitable for parenteral administration in human beings and mammals.

Summary of the invention

Accordingly, the present invention relates to a process for preparation of a low toxicity, stable oxazaphosphorine-containing composition comprising an oxazaphosphorine antineoplastic, mesna and an etherified β-cyclodextrin; the process comprising the steps of:

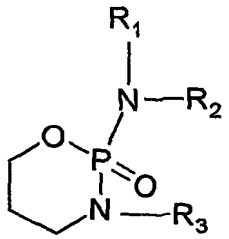
i) adding the oxazaphosphorine antineoplastic to an aqueous solution of an etherified β-cyclodextrin;

ii) adding mesna as such or as an aqueous solution optionally containing an etherified β-cyclodextrin to the oxazaphosphorine solution of step (i); and

iii) mixing the resultant aqueous solution and, optionally, making up the volume with water.

Detailed description of embodiments of the Invention

Oxazaphosphorine antineoplastic used in the invention is of the formula:



in which at least two of R_1, R_2 and R_3 independently are 2-chloroethyl and the remaining R radical is hydrogen. More preferably, the oxazaphosphorine antineoplastic is Cyclophosphamide ($R_1 = R_2$ = chloroethyl & R_3 = hydrogen) or, especially, Ifosfamide ($R_1 = R_3$ = chloroethyl & R_2 = hydrogen).

The etherified β-cyclodextrin preferably has at least some of the hydroxy groups etherified with hydroxyalkyl groups and optionally others etherified with alkyl groups and a water-solubility of more than about 1.8 g/100 ml water. Preferably the hydroxyalkyl groups are hydroxyethyl, dihydroxypropyl or, especially, hydroxypropyl groups and the alkyl groups, if present, are methyl or ethyl groups; The molar substitution (MS) by hydroxyalkyl groups (calculated as moles of alkylating alkylene oxide per anhydroglucose unit) suitably is about 0.05 to about 10, preferably about 0.2 to about 2, and especially about 0.5 to about 1.2.

The oxazaphosphorine antineoplastic content of the composition usually is from about 1 mg/ml to about 1000 mg/ml, preferably from about 25 mg/ml to about 750 mg/ml, and more preferably from about 50 mg/ml to about 500 mg/ml.

The ratio of oxazaphosphorine antineoplastic to mesna is usually in the range of about 20 : 1 to about 1 : 2 on a weight basis, preferably in the range of about 10 : 1 to about 1 : 1 on a weight basis.

The content of etherified β-cyclodextrin in the composition usually is from about 1% to about 60% w/v, preferably about 2.5% to about 40% w/v, more preferably about 5% to about 20% w/v.

Conventional parenteral additives may be present in the aqueous solution to which the oxazaphosphorine antineoplastic is added and/or in the aqueous solution to which mesna is added. These additives may also be added separately as a solution in water either before adding Mesna to oxazaphosphorine solution or before making up the volume. Such additives can be, for example buffers, isotonic diluents, anticrystallising agents, sequestering agents, or antioxidants as commonly used in aqueous parenteral compositions.

Buffers are selected from pharmaceutically acceptable buffer systems such as, for example, phosphate buffer, citrate buffer, glycine buffer containing any of the commonly used compounds or a mixture of compounds selected from citric acid, sodium citrate, potassium citrate, glycine, phosphoric acid, sodium phosphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium phosphate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, sodium hydroxide, potassium hydroxide, hydrochloric acid. Preferably the buffer used is a mixture of sodium dihydrogen phosphate and disodium hydrogen phosphate.

The aqueous solutions are mixed, preferably by intimate stirring and the resultant solution is usually sterilized by filtering through a sterilising grade filter. Preferably, the solution is filtered through 2μ and 0.2μ filters successively or just through a 0.2μ filter.

Usually, the filtrate will be aseptically filled into sterile containers such as vials, ampoules, plastic containers and sealing the filled containers.

The invention will now be illustrated by way of Examples. These Examples are by way of illustration only and in no way restrict the scope of the invention.

Ifosfamide used in these Examples was of parenteral grade complying with US Pharmacopoeial specifications. Mesna used in these Examples was of parenteral grade. Hydroxypropyl Beta Cyclodextrin (HPBCD) used was manufactured by Wacker Chemie having degree of substitution per glucose unit by alkyl groups between 0.5 to 1.2. Equipments used were of conventional nature; the entire processing was done in an area with a controlled environment. Water used in these Examples was of parenteral grade complying with "Water for Injection" specifications. All other additives used in these Examples were of parenteral grade.

Example I:

1.	Ifosfamide	10g
2.	Mesna	2g
3.	HPBCD	40g
4.	Disodium hydrogen phosphate	0.1g
5.	Sodium dihydrogen phosphate	0.06g
6.	Water	q.s. to 200 ml

Weighed quantities of disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in 160 ml of water and a weighed quantity of HPBCD was added and dissolved slowly under stirring. The resultant HPBCD solution was divided into two equal parts.

A weighed quantity of Ifosfamide was gradually added under stirring to one part of buffered HPBCD solution and mixed well.

A weighed quantity of Mesna was gradually added under stirring to the remaining part of buffered HPBCD solution and mixed well.

Mesna solution prepared above was added to Ifosfamide solution. The resulting solution was mixed together. The volume was made up to 200 ml with water. The product was filtered through a 0.2μ filter and filled aseptically in sterile glass vials. The glass vials were closed under aseptic conditions with sterile Teflon™ coated rubber bungs and sealed using flip off seals.

The composition obtained in this Example was analysed for Ifosfamide content and Mesna content by high pressure liquid chromatography (HPLC) and was found to contain 52.92 mg/ml of Ifosfamide and 10.2 mg/ml of Mesna. The composition had a pH of 6.86.

Example II:

The composition obtained in Example I was subjected to acute toxicity studies in mice. A conventional formulation, Holoxan™ manufactured by M/s. German Remedies was reconstituted as directed by the manufacturer and was used as a control after mixing with Mesna (equivalent to 20% of Ifosfamide content). Both the drug solutions were suitably diluted with 5% Dextrose Injection and administered intravenously. Ifosfamide in the doses of 500 mg/kg, 700 mg/kg and 900 mg/kg body weight was administered in three different groups of animals, each group consisting of eight animals.

The animals were kept under observation for 14 days and mortality recorded at the end of 3 days and 7 days.

It was observed that the LD_{50} dose was higher for composition of Example I in comparison with the Conventional formulation.

Composition of Example I			**Conventional formulation**		
	Mortality (%)			***Mortality (%)***	
Dose (mg)	3 Days	7 Days	***Dose (mg)***	3 Days	7 Days
500	0	0	500	50	75
700	0	50	700	100	100
900	75	100	900	100	100
LD_{50}	**700 – 900**	**700**	**LD_{50}**	**500**	**<500**

The above data clearly indicates that composition of Example I is less toxic compared to the Conventional formulation.

Example III:

1.	Ifosfamide	10g
2.	Mesna	2g
3.	HPBCD	20g
4.	Disodium hydrogen phosphate	0.1g
5.	Sodium dihydrogen phosphate	0.06g
6.	Water	q.s. to 200 ml

Weighed quantities of disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in 160ml of water and a weighed quantity of HPBCD was added and dissolved slowly under stirring.

A weighed quantity of Ifosfamide was gradually added under stirring to the buffered HPBCD solution and mixed for 3 hours.

After 3 hours, a weighed quantity of Mesna was gradually added under stirring to the buffered Ifosfamide solution. The volume was made up to 200 ml with water and filtered through a 0.2μ filter and filled aseptically in sterile glass vials. The glass vials were closed under aseptic conditions with sterile Teflon™ coated rubber bungs and sealed using flip off seals.

Example IV:

1.	Ifosfamide	10g
2.	Mesna	2g
3.	HPBCD	80g
4.	Disodium hydrogen phosphate	0.1g
5.	Sodium dihydrogen phosphate	0.06g
6.	Water	q.s. to 200 ml

The procedure of Example I was repeated using the components in the amounts set forth above.

Example V:

1.	Ifosfamide	10g
2.	Mesna	6g
3.	HPBCD	20g
4.	Disodium hydrogen phosphate	0.1g
5.	Sodium dihydrogen phosphate	0.06g
6.	Water	q.s. to 200 ml

The procedure of Example I was repeated using the components in the amounts set forth above.

Example VI:

1.	Ifosfamide	10g
2.	Mesna	16g
3.	HPBCD	20g
4.	Disodium hydrogen phosphate	0.1g
5.	Sodium dihydrogen phosphate	0.06g
6.	Water	q.s. to 200 ml

The procedure of Example I was repeated using the components in the amounts set forth above.

Example VII:

1.	Ifosfamide	100g
2.	Mesna	20g
3.	HPBCD	10g
4.	Water	q.s. to 200 ml

Weighed quantity of HPBCD was dissolved in 20ml of water. Ifosfamide was added gradually to HPBCD solution under stirring. Mixing was continued till a clear solution was obtained. Mesna was added gradually under stirring to the resultant Ifosfamide solution and mixed well till the entire quantity of Mesna went into solution. Volume was made upto 200ml with water.

The composition obtained in this Example was analysed for Ifosfamide content and Mesna content and was found to contain 497.88mg/ml of Ifosfamide and 98.73mg/ml of Mesna.

Example VIII:

1.	Ifosfamide	100g
2.	Mesna	60g
3.	HPBCD	10g
4.	Water	q.s. to 200 ml

Weighed quantity of HPBCD was dissolved in 20ml of water. Ifosfamide was added gradually to HPBCD solution under stirring. Mixing was continued till a clear solution was obtained. Mesna was added gradually under stirring to the resultant Ifosfamide solution and mixed well till the entire quantity of Mesna went into solution. Volume was made upto 200ml with water.

The composition obtained in this Example was analysed for Ifosfamide content and Mesna content and was found to contain 492.02mg/ml of Ifosfamide and 296.18mg/ml of Mesna.

Example IX:

1.	Ifosfamide	100g
2.	Mesna	60g
3.	HPBCD	10g
4.	Disodium hydrogen phosphate	0.8g
5.	Sodium dihydrogen phosphate	0.44g
6.	Disodium edetate	0.01g
7.	Water	q.s. to 200 ml

HPBCD was dissolved in 20ml of water. Ifosfamide was then added gradually to HPBCD solution under stirring. Mixing was continued till a clear solution was obtained. Mesna was added gradually under stirring to the resultant Ifosfamide solution. Disodium edetate, disodium hydrogen phosphate and sodium

dihydrogen phosphate were dissolved in 10ml of water and was added to Ifosfamide-Mesna solution and mixed well. Volume was made upto 200ml with water.

Example X:

1.	Ifosfamide	180g
2.	Mesna	36g
3.	HPBCD	10g
4.	Water	q.s. to 200 ml

The procedure of Example VIII was repeated using the components in the amounts set forth above.

Example XI:

1.	Ifosfamide	10g
2.	Mesna	2g
3.	HPBCD	20g
4.	Disodium hydrogen phosphate	0.1g
5.	Sodium dihydrogen phosphate	0.06g
6.	Water	q.s. to 200 ml

Weighed quantities of disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in 160 ml of water and a weighed quantity of HPBCD was added and dissolved slowly under stirring. Weighed quantity of Ifosfamide was gradually added under stirring to buffered HPBCD solution and mixed for 3 hours.

Weighed quantity of Mesna was gradually added under stirring to the above Ifosfamide solution and mixed well.

Volume was made up to 200 ml with water. The product was filtered through a 0.2μ filter and filled aseptically in sterile glass vials. The glass vials were closed under aseptic conditions with sterile Teflon™ coated rubber bungs and sealed using flip off seals.

The composition obtained in this Example was analysed for Ifosfamide content and Mesna content and was found to contain 51.2mg/ml of Ifosfamide and 10mg/ml of Mesna. The composition had a pH of 7.05.

Example XII:

The composition obtained in Example XI along with conventional formulation Holoxan™ manufactured by M/s. German Remedies were subjected to Hemorrhagic cystitis studies in rats to evaluate the bladder toxicity.

Experimental details are as follows:

Animals used	: Wistar rats of either sex.
Weight range of animals	: 100-150 gm.
Number of groups	: 5
Number of animals per group	: 2
Acclimatization	: One week under test conditions under controlled temperature and humidity.
Test Materials	: ***Ifosfamide with Mesna Injection***
Identity	: Composition of Example XI
Description	: Clear colourless solution
Route of administration	: Intravenous
Comparative material	:***Holoxan™***
Identity	:Ifosfamide injection U.S.P.
Lot No.	:G 220

Manufacturing Date	:October 2001
Expiry Date	:September 2003
Description	:Dry powder for reconstitution with water for injection
Strength	:40 mg/ml on reconstitution
Manufacturer	: German Remedies Limited.
Route of administration	: Intravenous

Study design

Animals were divided into 5 groups and each group comprised two animals. The animals received injections of Ifosfamide formulations as specified in table 1.

Table 1.Doses of Ifosfamide Formulations.

Group No.	Formulation	Dose (mg/kg body weight)	
		Ifosfamide	Mesna
1	Holoxan	400	80
2	Holoxan	500	100
3	Example XI	400	80
4	Example XI	500	100
5	Dextrose Inj.	-	-

All animals received injections via the intravenous route. The animals were sacrificed 24 hours after injection. The urinary bladder of all the animals were collected and was fixed in 10% formalin for 48 hours. Histopathological slides of the organ were prepared and subjected to microscopic examination.

EVALUATION: Table 2 represents the grading pattern for the hemorrhagic cystitis.

Table 2 : Grading pattern for hemorrhagic cystitis.

Grading	Score
Normal	0 (N)
Mild hemorrhagic cystitis	1 +
Moderate hemorrhagic cystitis with or without epithelial atypia	2 +
Severe hemorragic cystitis with or without epithelial atypia	3 +

OBSERVATIONS: Table 3 depicts the evaluation results on hemorrhagic cystitis of two formulations of Ifosfamide

Table 3 : Evaluations of two formulations of Ifosfamide for hemorrhagic cystitis.

Animal No.	Formulation	Dose (mg/kg)		Score
		Ifosfamide	Mesna	
1.	Holoxan	400	80	1 +
2.	Holoxan	400	80	1 +
3.	Holoxan	500	100	1 +
4.	Holoxan	500	100	2 +
5.	Example XI	400	80	N
6.	Example XI	400	80	N
7.	Example XI	500	100	N
8.	Example XI	500	100	N
9.	Dextrose	-	-	N
10	Dextrose	-	-	N

DISCUSSION:

Animals treated with Holoxan showed hemorrhagic cystitis at both doses of 400mg/kg and 500mg/kg whereas composition of Example XI did not show hemorrhagic cystitis.

CONCLUSION:

The above findings conclusively proved that the composition of Example XI is less toxic than the conventional formulation Holoxan™

Example XIII:

The composition obtained in Example XI was subjected to stability studies. The data is as follows :

Storage condition	Description	Ifosfamide content
Initial	Clear, colourless liquid	51.2mg/ml
2°C-8°C – 3M	Clear, colourless liquid	50.06mg/ml
2°C-8°C – 6M	Clear, colourless liquid	50.33mg/ml

Conclusion:

From the above it is evident that Ifosfamide is stable in the composition obtained in Example XI without undergoing any degradation when stored at 2°C – 8°C whereas the conventional formulation on reconstitution is reported to be stable for three to six weeks under refrigeration.

The advantages of the invention:

1. The composition of present invention is ready-to-use, stable and has low toxicity.
2. Formulating Ifosfamide in aqueous solution with Mesna for parenteral administration provides ease of handling the cytotoxic drug as no reconstitution of the powder formulation is required.
3. The content of Ifosfamide can be increased to as high as 10g in a 10ml vial as against 1 to 2g in a conventional marketed packs. This reduces the number of containers to be handled during administration of the drug.
4. No additional step of mixing with Mesna is required as the composition of present invention is formulated as a solution of Ifosfamide with Mesna.

CLAIMS

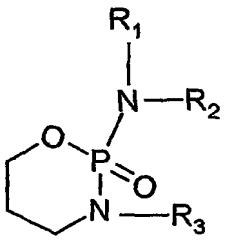
1. A process for preparation of a low toxicity, stable oxazaphosphorine-containing composition comprising an oxazaphosphorine antineoplastic, mesna and an etherified β-cyclodextrin; the process comprising the steps of:

   i) adding the oxazaphosphorine antineoplastic to an aqueous solution of an etherified β-cyclodextrin;

   ii) adding mesna as such or as an aqueous solution optionally containing an etherified β-cyclodextrin to the oxazaphosphorine solution of step (i); and

   iii) mixing the resultant aqueous solution and, optionally, making up the volume with water.

2. A process as claimed in Claim 1, wherein the oxazaphosphorine antineoplastic is of the formula:



in which at least two of R_1, R_2 and R_3 independently are 2-chloroethyl and the remaining R radical is hydrogen.

3. A process as claimed in Claim 2, wherein the oxazaphosphorine antineoplastic is Cyclophosphamide (R_1 = R_2 = chloroethyl & R_3 = hydrogen).

4. A process as claimed in Claim 2, wherein the oxazaphosphorine antineoplastic is Ifosfamide (R_1 = R_3 = chloroethyl & R_2 = hydrogen).

5. A process as claimed in any one of the preceding claims, wherein the etherified β-cyclodextrin used is Hydroxypropyl Beta Cyclodextrin (HPBCD).

6. A process as claimed in Claim 5, wherein the molar substitution of HPBCD is from about 0.5 to about 1.2.

7. A process as claimed in any one of the preceding claims, wherein the oxazaphosphorine antineoplastic content of the composition is from about 1 mg/ml to about 1000 mg/ml.

8. A process as claimed in Claim 7, wherein said oxazaphosphorine antineoplastic content is from about 25 mg/ml to about 750 mg/ml.

9. A process as claimed in Claim 8, wherein said oxazaphosphorine antineoplastic content is from about 50 mg/ml to about 500 mg/ml.

10. A process as claimed in Claim 9, wherein said oxazaphosphorine antineoplastic content is about 50 mg/ml.

11. A process as claimed in Claim 9, wherein said oxazaphosphorine antineoplastic content is about 500 mg/ml.

12. A process as claimed in any one of the preceding claims, wherein the ratio of oxazaphosphorine antineoplastic to mesna is in the range of about 20 : 1 to about 1 : 2 on a weight basis.

13. A process as claimed in claim 12, wherein the ratio of oxazaphosphorine antineoplastic to mesna is in the range of about 10 : 1 to about 1 : 1 on a weight basis.

14. A process as claimed in claim 13, wherein the ratio of oxazaphosphorine antineoplastic to mesna is 10 : 2 on a weight basis.

15. A process as claimed in claim 13, wherein the ratio of oxazaphosphorine antineoplastic to mesna is 10 : 6 on a weight basis.

16. A process as claimed in any one of the preceding claims, wherein the content of etherified β-cyclodextrin in the composition is about 1% to about 60% w/v.

17. A process as claimed in Claim 16, wherein said etherified β-cyclodextrin content is about 2.5% to about 40% w/v.

18. A process as claimed in Claim 17, wherein said etherified β-cyclodextrin content is about 5% to about 20% w/v.

19. A process as claimed in any one of the preceding claims, wherein one or more conventional parenteral additives are incorporated into the aqueous solution of Claim 1 step (i) or Claim 1 step (ii) or in water used for making up the volume in Claim 1 step (iii).

20. A process as claimed in any one of the preceding claims, wherein said mixture of resultant aqueous solutions is sterilized by filtering through a sterilising grade filter.

21. A process as claimed in Claim 20, wherein the filtrate from the sterilising grade filter is aseptically filled into sterile containers and the filled containers are sealed.

22. A process as claimed in Claim 1 and substantially as herein before described with reference to any of the Examples.

23. A stable oxazaphosphorine-containing composition obtainable by a process as claimed in any one of the preceding claims.

24. A stable oxazaphosphorine-containing composition prepared by a process as claimed in any one of the preceding claims.

25. The use of a stable oxazaphosphorine-containing composition as defined in Claim 23 or Claim 24 in the manufacture of a medicament for the treatment of malignant disease.

26. A method of treating a malignant disease comprising administering to a patient suffering said disease an effective amount of a sterile stable oxazaphosphorine-containing composition as defined in Claim 23 or Claim 24.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: LIQUID STABLE COMPOSITION OF OXAZAPHOSPHORINE WITH MESNA

(57) Abstract: A low toxicity, stable oxazaphosphorine containing compositions with mesna for parenteral administration has been described. The process essentially requires addition of an oxazaphosphorine antineoplastic to the aqueous solution of an etherified b-cyclodextrin followed by addition of mesna as such or as an aqueous solution containing optionally, an etherified b-cyclodextrin. Preferably, the oxazaphosphorine antineoplastic is Ifosfamide and the etherified b-cyclodextrin is 2-hydroxypropyl-b-cyclodextrin.

WO 2004/022699 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN03/00298

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/675, 31/185, 31/724; A61P 35/00
US CL : 514/711, 712, 709, 54, 57, 60, 79, 89,80, 137; 424/422

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/711, 712, 709, 54, 57, 60, 79, 89,80, 137; 424/422

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 91/04026 A1 (AUSTRALIAN COMMERCIAL RESEARCH & DEVELOPMENT LIMITED) 04 April 1992 (04.04.91), see entire documents, especially page 7, lines 14-19; page 8, lines 4-23; claims.	2-4
Y	WO 03/002101 A1 (FARMATRON LTD) 09 January 2003 (09.01.2003), see entire documents, especially page 6, lines 5-9; page 7, line 11; claims.	2-4
Y	WO 96/30024 A1 (INSTYTUT FARMACETUTYCZNY) 03 October 1996 (03.10.1996), see entire documents, especially page 2, lines 3-14.	2-4
Y	US 4,959,215 A (SAUERBIER et al) 25 September 1990 (25.09.1990), see entire documents.	2-4
Y	EP 0 895 783 A2 (ASTA MEDIA AKTLENGESELLSCHAFT) 10 February 1999 (10.02.1999), see entire documents.	2-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Intern[redacted] application No.
PCT/IN03/00298

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 1 and 5-26
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Please See Continuation Sheet

3. ☒ Claim Nos.: 7-21, 23-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Continuation of Box I Reason 2:
Claims 1 and 5-26 relate to an extremely large number of composition comprising compounds having characteristic of oxazaphosphorine. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compositions or compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely oxazaphosphorine antineoplastic of the formula in claim 2.

Continuation of B. FIELDS SEARCHED Item 3:
STN ONLINE
search terms: ifosfamide, mesna, cyclodextrin